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In re Application of:	)	Art Unit: 1648
Ashok AMIN, et al.	)	Examiner: D.C. WORTMAN
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For: METHOD FOR TREATING HEPATITIS	)	

Declaration Under Rule 3.312

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We, Ashok Amin, M.D. and Steven Abramson, M.D., do hereby declare that we are inventors of the above-identified application.

TNF-alpha mediates its effect via two distinct high affinity TNF receptors (TNFR), p55 and p75. They are similar in their extracellular domains but have distinct intracellular domains and different distributions. TNFRs on the cell surface are cleaved by proteolytic enzymes and released into circulation. An increase in these serum soluble TNFRs (sTNRF) are believed to reflect enhanced expression of these receptors on various cells, and increased activation of the TNF-alpha system. The role of sTNFR is still unclear. Some authors have suggested that the effect of

sTNFR may be concentration dependent. *In vitro*, at low concentrations it may act to stabilize the activity of TNF-alpha, and at high concentrations it may act to inhibit TNF-alpha.

Etanercept is a dimeric fusion protein of the extracellular ligand-binding protein of the human tumor necrosis factor receptor (p75) linked to the Fc portion of human IgG1. Etanercept binds specifically to tumor necrosis factor (TNF) and blocks its interaction with cell surface receptors.

There have been numerous studies indicating that the cytokine system plays an important role in the pathogenesis of hepatitis C infection with respect to both severity and chronicity. TNF-alpha is a cytokine secreted mainly by activated macrophages and lymphocytes and is thought to have a wide array of effects including antineoplastic, antiviral, and immunomodulatory properties. The hepatitis C virus has been reported to induce TNF alpha gene expression and TNF alpha itself. In patients with chronic hepatitis C infection, cytotoxic T-lymphocytes are thought to play a major role in the production of TNF alpha. In addition, TNF alpha has been shown to induce hepatitis when injected into humans and rodents.

TNF alpha may play a key role in the recruitment of monocytes, macrophages, lymphocytes, and the expression of adhesin molecules. It is thought to be pivotal in the inflammatory process, and may mediate apoptosis of hepatitis C infected hepatic cells as well as "bystander" cells. It is quite possible that the hepatitis C virus requires the TNF receptor as one of the co-receptors, as observed in other viral infections. The hepatitis virus has been reported to induce TNF alpha-dependent apoptosis in hepatocytes while binding through viral core proteins. TNF alpha and the hepatitis C core proteins together may be required to induce apoptosis in hepatocytes. Etanercept may inhibit to co-signal generated by TNF-alpha via the death domain of its receptor for hepatic apoptosis and infection process. In contrast, an article by Ray et al. in 1998 reports that hepatitis C core protein inhibits TNF-alpha induced apoptosis.

In this way, TNF-alpha may be an important determinant of activity and chronicity of hepatitis C infection. It should be noted that interferon alpha 2b, which is one of the therapeutic agents for hepatitis C, upregulates soluble TNF-alpha receptors following the first dose of recombinant interferon alpha 2b. In other studies, the sustained responders to treatment with

interferon alpha 2b showed a significant decrease in the levels of soluble TNFRp75 but not p55 compared to pretreatment levels.

Previous studies have shown that there is an upregulation of TNF-alpha and TNF-alpha receptors p55 and p75 in patients with chronic hepatitis C infection (Kallinowski, 1997). The levels of TNFR appear to correlate with the severity of liver disease in the patients (Kallinowski, 1997 and Itoh, 1999). Specifically, TNFRp75 has been reported to have significant correlations with serum levels of AST, ALT, GGT, and gamma globulin in patients with chronic hepatitis C (Itoh, 1999).

Additionally, in support of the theory that anti-TNF therapy may have a role in the treatment of hepatitis C infection, Sekimoto et al., in 2000, reported in an animal model that pretreatment with anti-TNF monoclonal antibodies prevented liver injury in mice.

The variable response of Etanercept on hepatitis C in the patients we have treated may be due to a variety of viral and host factors, including polymorphisms of the TNF-alpha promoter gene at position -238 and -308. Polymorphisms in the TNF-alpha gene have been reported to be associated with hepatitis C infection (Hohler, Kruger, 1998). TNF-alpha promoter gene

polymorphisms were noted as an important factor in the variability of the severity of hepatitis C recurrence after liver transplantation (Rosen, 1999) and could also play a role in the variable responses to Etanercept. Additional possibilities include differences in hepatitis C genotypes and host HLA genotypes.

Under our direction and control, patients with chronic hepatitis C infection were treated with anti-tumor necrosis factor (TNF) therapy. Our clinical observations suggest that TNF plays a provocative but elusive role in the pathophysiology of hepatitis C.

MK (Patient 1) is a 59 year old woman with a six year history of polyarticular rheumatoid arthritis. Routine laboratory examination revealed evidence of hepatitis characterized by chronic and persistent elevations of transaminases (AST, ALT) in the range of 100-200 units. Further evaluation noted a serum hepatitis C viral RNA (HCV RNA) elevated at >1,000.000 copies/ml on repeated occasions. A liver biopsy in 1997 revealed chronic active hepatitis. The patient MK did not receive any treatment for her hepatitis C infection.

In May 2000, the patients was started in Etanercept 25 mg subcutaneous injections twice weekly as treatment for her rheumatoid arthritis that was not responsive to other medications. The therapy resulted in

a modest improvement of her synovitis. Remarkably, however, laboratory tests revealed normalization of her transaminases (AST, ALT) to < 50 units within a few months of initiating Etanercept treatment. In addition, the HCV RNA fell to 165,000 copies/ml. These improvements persisted over four months of follow up.

Since the treatment of the patient described above, we became aware of seven additional patients who ha received Etanercept for the treatment of rheumatoid arthritis, who had concomitant hepatitis C infection.

Of these seven patients, described in Table 1 as patient 2-8, Patients 2 and 3 had marked (>75%) decrease in HCV RNA following Etanercept therapy. Only Patient 2 had elevated transaminases prior to therapy; these tests normalized following treatment with Etanercept. This same patient also had a marked increase of the HCV RNA following cessation of Etanercept therapy.

The remaining six patients either showed no response or had inadequate data for assessment.

A summary of the finding in the three patients whose HCV RNA responded to treatment with Etanercept is shown in Table 1. The remaining patients' results are shown in Table 2.

Table 1

	HCV RNA, PCR (copies/ml)	AST (units/L)	ALT (units/L)	Liver Biopsy	Genotype
Patient 1				1997- chronic active hepatitis 1999- chronic hepatitis with bridging fibrosis and nodule and formation, moderate steatosis	1b
1/00	985,000	137	116		
3/00		100	102		
	>1,000,000	100-200	100-200		
5/00 Etanercept started					
7/00	165,000	177	124		
9/00	121,000	48	53		
11/00	130,000	156	171		
Etanercept discontinued					
Patient 2				1993- mild nonspecific lymphocytic portal inflammation and minimal fatty change	?
11/96	366,000	58	39		
12/98	241,000	68	37		
12/98 Etanercept started					
2/99	211,000	N/A	36		
5/99	2829	26	36		
8/99	798	38	36		
9/99	989	34	38		
Etanercept discontinued by patient					
12/99	>1,000,000	37	40		
2/00	>1,000,000	44	38		
Patient 3				1994- chronic hepatitis C	1b
12/98	2733	34	28		
12/98 Etanercept started					
1/99	27.7	38	40		
3/99	37	37	35		
6/99 Etanercept discontinued due to rash					
7/00	detected		38		

Table 2

	HCV RNA, PCR (copies/ml)	AST (units/L)	ALT (units/L)	Liver Biopsy	Genotype
Patient 4				1998- chronic hepatitis C	?
3/98	443,309	35	42		
9/99	14.5	37	32		
10/99 Etanercept started					
4/00	>1,000,000	39	32		
8/00	detected (not quantitated)	40	35		
9/00 Etanercept discontinued					
Patient 5				?	1a
5/98	197	38	57		
7/99	851	N/A	N/A		
10/99 Etanercept started					
11/99	790,000	37	49		
12/99	>1,000,000	84	47		
2/00	>1,000,000	38	43		
7/00	120,000	N/A	N/A		
Patient 6				1998- chronic hepatitis C, moderate activity, mild to moderate fibrosis	?
7/98	>1,000,000	256	97		
9/99	N/A	290	N/A		
11/99	131,000	33	47		
3/00 Etanercept started					
4/00	345,000	70	99		
7/00	detected (not quantitated)	71	130		
8/00	pending	47	65		
Patient 7				?	?
3/99	314	55	N/A		
11/99 Etanercept started					
12/99	967,000	52	107		
5/00	>1,000,000	63	73		
9/00	>1,000,000	43	79		
Patient 8				?	?
9/98	<0.5	15	14		
3/99 Etanercept started					
7/99	<2000	15	11		
9/99	<2000	14	10		
5/00	<2000	11	10		

There are now some published reports supporting the effectiveness of anti-TNF-alpha therapy in hepatitis patients, for example, Tilg H., Jalan R. et A. et al. (2003) Anti-tumor factor-alpha monoclonal antibody therapy in severe alcoholic hepatitis *J Hepatol* 38: 419-25 and Zylberberg H., Rinaniol AC, Pol S, et al. (1999) soluble tumor necrosis factor receptors in chronic hepatitis C: A correlation with histological fibrosis and activity *J Hepatol* 30: 185-191.

It should also be noted that the original label on TNF-alpha inhibitors warned that these inhibitors not be used in patients with infections.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 81 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon:

Ashok Amin, M.D. Steven B. Abramson, M.D.